the initial 11 *Kelletia kelletii* were tagged on December 11, 1967. Success in finding these motile benthonic animals is largely dependent upon the visibility underwater, although searching from a permanently emplaced survey line eases the difficulty in locating them.

Other mollusks have been successfully tagged using this method: The rock scallop, *Hinnites multirugosus* (Gale, 1928); the red abalone, *Haliotis rufescens;* and the wavy top shell, *Astraea undosa* (Wood, 1828).


Figure 1

The drawing shows a tagged *Kelletia kelletii* with a numbered disc embedded in epoxy and cemented to the dorsal region of the shell.

The size of the mollusk does not present a problem, since the epoxy can be shaped to conform to the characteristics of the outer shell and the disc tags sized accordingly. This reduces any hindrance to the movement of the animal and the possibility of the epoxy coming in contact with the animal's living tissue (Figure 1).

The advantages of this technique appear to be the permanence of marking, and the fact that tagging can be completely carried out underwater with a minimum amount of disturbance to the subjects and their environment.

## ACKNOWLEDGMENTS

I am particularly grateful to Dr. W. D. Clarke for valuable advice and encouragement, and to Mr. R. E. Bower for technical assistance in the field.

## LITERATURE CITED

Cox, Keith W.
1962. California abalones, family Haliotidae. Fish Bull. 118, Calif. Dept. Fish and Game, Sacramento, Calif.

Forster, G. R.
1967. The growth of *Haliotis tuberculata*: results of tagging experiments in Guernsey 1963-65. Journ. Marine Biol. Assoc. U. K. 47: 287 - 300

Newman, G. G.
1966. Movements of the South African abalone *Haliotis midae*. Invest. Rep. Div. Sea Fish. S. Afr. No. 56: 1 - 20

Randall, John E.
1964. Contributions to the biology of the queen conch, *Strombus gigas*. Bull. Mar. Sci. Gulf and Carib. 14 (2): 246 to 293

# A Method of Color Preservation in Opisthobranch Mollusks

BY

GORDON A. ROBILLIARD

Department of Zoology
University of Washington
Seattle, Washington 98105

## INTRODUCTION

Color and color pattern are useful taxonomic characters in the opisthobranch mollusks. However, I know of no published reports describing a method of preserving color in opisthobranchs. Instead, it is usually assumed that the colors will fade or be bleached out in formalin,

alcohol, or other preservatives. Color is usually preserved in color photographs or field notes or both.

Toyama & Miyoshi (1963) and Waller & Eschmeyer (1965) successfully employed an antioxidant, butylated hydroxytoluene (hereafter referred to as Ionol C. P. -40), to preserve some colors in fish and a prawn. Waller & Eschmeyer (*op. cit.*) used 1, 10, and 20 cc of Ionol C. P. -40 per 4500 cc of 10% formalin to make the test solutions (0.02%, 0.22%, and 0.44% concentrations respectively). Eighteen months later, they found that the color of fish in the 0.44% solution was best preserved, but preservation of colors of fish in other test solutions was superior to that of fish in untreated formalin solution.

In this note, I report the results of testing this technique on opisthobranchs from 3 orders (Cephalaspidea, Sacoglossa, and Nudibranchia) with emphasis on the nudibranchs.

## MATERIALS AND METHODS

A stable emulsion was formed by vigorously stirring Ionol C. P. -40 into hot sea water (55° to 65° C) (Shell Technical Bulletin, IC:67-16). Concentrations of Ionol C. P. -40 used in the emulsions gave final dilutions of 0.1% to 0.5% Ionol C. P. -40 by volume. Formalin was added to this emulsion to give a final concentration of 5% formalin by volume (hereafter referred to as Ionol C. P. -40 emulsion). Ionol C. P. -40 is also readily soluble in alcohol.

Most of the opisthobranchs were relaxed for 1 to 5 minutes, depending on size, with succinylcholine chloride (Beeman, 1968). A few, especially the Cephalaspidea, were relaxed for 2 to 8 hours in propylene phenoxetol using 1% by volume of propylene phenoxetol in sea water (Owen, 1955; Owen & Steedman, 1958).

Small animals ($< 1.5$ to 2.0 cm long) were then put directly into Ionol C. P. -40 emulsion. Formalin diffuses inward rapidly enough to preserve the internal tissues. Larger animals ($> 2$ cm long) were injected with a small amount of 5% formalin in sea water without Ionol C. P. -40, to preserve the internal tissues, then put into the Ionol C. P. -40 emulsion.

The Ionol C. P. -40 emulsion was not injected into the animal because it eventually forms an oily film in the body cavities and over the tissues. This is particularly undesirable if the animals are to be dissected. In any case, there are few internal organs in an opisthobranch in which color is distinctive or taxonomically important.

The method was tested on 1 sacoglossan, *Elysia hedgpethi* Marcus, 1961; 2 cephalaspideans, *Haminoea* sp., and *Aglaja diomedea* (Bergh, 1893); and 30 species of nudibranchs of which 12 were dorids, 12 were dendronotaceans, and 6 were aeolids.

## RESULTS

Of the concentrations tested, 0.3% Ionol C. P. -40 emulsion resulted in maximal color retention and was used for most of the specimens.

Stored in bottles in glass-door cabinets, the specimens in Ionol C. P. -40 emulsion have been exposed to normal artificial lighting, but protected from direct sunlight, for up to 2 years. In most cases, the original color has been retained albeit with varying degrees of fading in some species. The color of virtually all specimens stored under similar conditions, but in untreated 5% formalin in sea water, has faded markedly or disappeared.

Some colors are preserved better and longer than others. The yellow body color and black spots of *Anisodoris nobilis* (MacFarland, 1905) and *Archidoris montereyensis* (Cooper, 1862) are well preserved as are the yellow and orange pigments of *Triopha carpenteri* (Stearns, 1873). The orange on the cerata of *Laila cockerelli* MacFarland, 1905 is completely preserved while the orange on *Hermissenda crassicornis* (Eschscholtz, 1831) faded slightly. Slight fading has occurred in the orange-red body color of *Rostanga pulchra* MacFarland, 1905. The salmon-pink body color of *Tritonia festiva* (Stearns, 1873) and *T. gilberti* (MacFarland, 1966) and the orange body color of *T. (Tochuina) tetraquetra* (Pallas, 1788) fade only slightly. The same is true of the brown blotches of *Diaulula sandiegensis* (Cooper, 1862). Many specimens of *Dendronotus* spp. have been preserved in Ionol C. P. -40 emulsion and the colors – white, metallic orange, brown, magenta, yellow, mauve, purple, pink, grey, red – have faded slightly over periods of up to 2 years. The orange body color and white spots of *Dirona aurantia* Hurst, 1965 and the white pigment on the cerata of *D. albolineata* Cockerell & Eliot, 1905 fade somewhat. The dark green body color of *Elysia hedgpethi* and the black (or dark purple) of *Aglaja diomedea* have faded very little.

In a few cases, the color faded markedly or completely disappeared. The chocolate brown color of *Onchidoris bilamellata* (Linnaeus, 1767) faded to about a third of its original intensity. In *Cadlina marginata* MacFarland, 1905, the yellow pigment disappears completely within 12 to 24 hours. The yellow on the notal papillae of *Acanthodoris hudsoni* MacFarland, 1905 fades markedly over a week. Structural colors, such as blue on *Hermissenda crassicornis* and other opisthobranchs (Bürgin,

1965, BÜRGIN-WYSS, 1961) usually disappear when the animal is killed and they cannot be preserved by an antioxidant.

## CONCLUSION

The use of an antioxidant such as Ionol C. P. -40 with a preservative seems to be a successful and relatively simple method of preserving most colors in the opisthobranchs. However, on the basis of this study, it is not possible to generalize about which colors will be preserved between different taxa or even within a certain taxon. Further experimentation with Ionol C. P. -40 will likely demonstrate a more widespread applicability as well as delineating more precisely which colors will or will not be preserved.

The optimal concentration proved to be 0.3% Ionol C. P. -40 by volume in a solution of 5% formalin in sea water.

Color retention is an obvious advantage to the taxonomist in that it allows him to augment the description of a species. This is especially true in soft-bodied animals like opisthobranchs where color, though quite variable intraspecifically, often serves as a guide in species identification.

## ACKNOWLEDGMENTS

I wish to extend my thanks to Brian Case, University of Manitoba, for first bringing the method to my attention and to the Shell Chemical Company, Industrial Chemicals Division (Portland Office) for the sample of Ionol C. P. -40. I am grateful to Dr. Alan J. Kohn for reading the manuscript and for his suggestions. This work was supported by a Special Scholarship from the National Research Council of Canada.

## LITERATURE CITED

ANONYMOUS
1967. Ionol CP antioxidant. Shell Tech. Bull. IC: 67-16

BEFMAN, ROBERT DAVID
1968. Order Anaspidea. The Veliger 3 (Supplement, prt. 2):87 - 102; plt. 11; 12 text figs. (1 May 1968)

BÜRGIN-WYSS, ULRIKE
1961. Die Rückenanhänge von *Trinchesia coerulea* (MONTAGU). Rev. Suisse Zool. 68 (4): 461 - 582; plt.

BÜRGIN, ULRIKE F.
1965. The color pattern of *Hermissenda crassicornis* (ESCHSCHOLTZ, 1831) (Gastropoda: Opisthobranchia: Nudibranchia) The Veliger 7 (4): 205 - 215; 9 text figs. (1 April 1965)

OWEN, GARETH
1955. Use of propylene phenoxetol as a relaxing agent. Nature 175 (4453): 434 (5 March 1955)

OWEN, GARETH & H. F. STEEDMAN
1958. Preservation of molluscs. Proc. Malacol. Soc. London 33 (3): 101 - 103

TOYAMA, KENZO & GOTA MIYOSHI
1963. Prevention from color fading of aquatic animals under preservation. Journ. Tokyo Univ. Fish. 50 (1): 43 - 48

WALLER, RICHARD A. & WILLIAM N. ESCHMEYER
1965. A method for preserving color in biological specimens. Bioscience 15 (5): 361

## BOOKS, PERIODICALS, PAMPHLETS

### Between Pacific Tides

by EDWARD F. RICKETTS & JACK CALVIN, Fourth edition, revised by JOEL W. HEDGPETH. September 19, 1968. xiv +614 pp.; 8 color plates; 302 text figures.

Stanford University Press, Stanford, California. $10.-.

This classic work on the ecology and natural history of plants and animals inhabiting the Pacific shores in the area between the low and the high tide marks was written, originally, "for laymen, for beginners . . . " but it has filled a need far beyond that envisioned by Ed Ricketts. Because of the thoroughly annotated bibliography, the book has become an important first source for many serious investigators and an indispensable text book in many courses in invertebrate zoology.

The book has gone through three previous editions, the first two by the original author, and the third edition revised by Dr. Hedgpeth. Each edition was better than the previous one, each updated to include the latest developments in the fields of endeavor covered by the scope of the work.

This fourth edition, again revised and brought up to date, is a worthy successor to the other three editions. Dr. Hedgpeth has brought his critical talents to bear and his influence can be perceived on practically every page, although he has managed beautifully to preserve the original charm of the book. And while a number of the illustrations are the old familiar ones, many have been superseded by better pictures illustrating more precisely what was desired to be called to the reader's attention. Even the color plates have been much improved, although they may perhaps, in a future edition, be printed with a still better technique which will render the delicate colors of the intertidal area more life-like.